REMARKS/ARGUMENTS

Pending Claims

Claims 4-8 and 12-16 are pending.

§ 112

Claims 4-8 and 12-16 stands rejected under 35 U.S.C. § 112 as allegedly non-enabling. Applicants respectfully traverse.

The Office Action's only basis for the § 112 rejection is that "there is nothing in applicant's written disclosure to teach or suggest how the enrichment procedure could be used to select subpopulations of the RET^+ cells populations with different particular lineage commitments for use." (Office Action, page 3, lines 7-10) Applicants disagree. Methods for selecting different subsets of cells are presented in page 23-25. In particular, the sole paragraph on page 23 shows how Applicants were able to determine the identify of proNPs cells in just 3 days. Table 2 on page 24 shows how Applicant was able to determine NP, ProNP, and NNP cells within 4 days by observation. Lines 10-17 of page 25 shows how NP cells were determined within 3-4 days of culture. Thus, the claimed invention is enabled and Applicants have disclosed at least one successful method of performing the invention in the specification.

In a sentence to support the rejection, the Office Action also alleges that "applicant admits that the three classes of RET^+ progenitor cells were indistinguishable 'by expression of any of the antigenic markers examined or by their morphology' " (Office Action, page 3, lines 10-12). Applicants believe that the Examiner has mischaracterized Applicant's statement. The statement quoted by the Examiner appears at the end of 3 pages of description (Specification, pages 23-25) showing that Applicants have performed the step of selecting for individual subpopulations using multiple methods. This statement, when viewed in context, is a statement of the shortcomings of the prior art and merely states that morphology is not a good method for identifying cells at that stage in development. The quote does not state or imply that there are no methods available to distinguish cell classes. For this reason, Applicants believe that the statement was improperly interpreted by the Examiner. As stated previously, Applicants have, in

fact, provided at least three pages of instructions showing multiple ways of identifying cells types immediately prior to the statement quoted by the Examiner.

Furthermore, the Office Action also alleges that "as a result of the cell culture, the desired cell subpopulation has already differentiated and been lost for selection and use" (Office Action, page 3, lines 16-17). Applicants believe this statement is in error because the methods shown by the Applicants (as discussed above on pages 23-25 of the specification) requires only 3 to 4 days for results. See, for example, page 23, lines 15-16 "In all cases, neuronal differentiation was detected morphologically within 3 days." Table 2 (page 24) showed the identity of NP, ProNP and NNP cells in an experiment that lasted only 4 days (see caption to Table 2). Identification of NP cells was performed within 3-4 days in another experiment (See, page 25, line 12). As shown in the specification, Applicants have reliably and consistently identified cell types within 3-4 days. Furthermore, the specification discloses that neural stem cells are capable of at least six to ten rounds of symmetric, self-renewing division (Page 32, lines 7-9). Thus, there is ample time for testing because six to ten rounds of cell division will take much longer than the 3 to 4 days needed to do the testing according to one embodiment of the invention. The alleged deficiency of the specification – that stem cells can differentiate before tests are finished - is in fact a limitation of the prior art that was solved by the methods of the invention.

For these reasons, the rejection of claims 4-8 and 12-16 under 35 U.S.C. § 112 as allegedly non-enabling was made in error and should be withdrawn.

§ 103

Claims 4-8 and 12-16 stand rejected under 35 U.S.C. § 103 as allegedly obvious in view of Lo et al., (Perspectives Dev. Neurobiol. 2:191-201, 1994), Stemple '93 (Stemple et al., Dev. Biol. 159: 12-23, 1993), Stemple '92 (Stemple et al Cell 71:973-985, 1992), and Martucciello et al. Applicants respectfully traverse.

It is the Examiner's position that the combination of references teaches the expression of RET as a valuable marker for very early stages in neural crest cell lineage diversification and suggest the isolation and culture of cells expressing the marker for further testing of developmental potential. Applicants disagree.

The claimed invention is directed to a method of enriching for a neural progenitor cell by combining neural crest derived cells with an anti-RET antibody and selecting for RET+ cells. The claimed invention is novel because it was the first time that it was recognized that RET is a valuable marker for isolating neural progenitor cells.

The cited references, Lo, Stemple '93, Stemple '92 and Martucciello, individually or in combination, do not render the claimed invention obvious because they do not disclose the usefulness of using RET as a marker for neural progenitor cells for at least two reasons. First, Lo is directed to MASH-1 as a marker for neural crest development. Lo does not describe an expression of the c-ret gene. For example, Lo states that "[i]t remains to be proven that c-ret and MASH- are expressed in the same cells. It is formally possible that the two genes are expressed in distinct but commingled cell populations." (Lo, at page 194). Lo admitted to the uncertainty by stating "It must be emphasized that MASH-1^+ and c-ret^+ cells could be different from each other, and that MASH-1^+ cells (or c-ret^+ cells) in different autonomic sublineages could be different. Definitive double-labeling experiments will be required to establish whether the genes are expressed in the same or in different cells." (Lo, at page 199). In fact, the authors in Lo have admitted that they have no idea how ret is regulated in neural cell development. See, for example, the legend to Figure 6 where the authors stated "This scheme postulated that neural crest cells undergo an early segregation into sensory, autonomic, and glial lineages and that the autonomic lineage is marked by expression of MASH-1. This scheme is purely speculative, for example, it is not clear whether progenitors committed to an autonomic fate can generate both neurons and glia or , as the diagram implies, only neurons." (Lo, at page 200).

Second, Stemple '93, Stemple '92 and Martucciello do not teach that RET is a useful marker for neural progenitor cells. Stemple '93 is a review of various environmental factors affecting growth and differentiation of neural crest development. Stemple makes references to using antibodies to cell surface antigens as a means of purifying subpopulations of cells within a mixture. Applicants have carefully reviewed Stemple '93 in its entirety and have not found a single reference to the RET antigen.

Stemple '92 refers to the alleged isolation of stem cells for neurons and glia from the neural crest using antibodies against the low affinity NGF receptor. Applicants have carefully reviewed Stemple '92 in its entirety and have not found a single reference to the RET antigen. Furthermore, Stemple is unsure that their stem cells are capable of developing into neurons. The neurons that developed from Stemple's stem cells were uncharacterized. See, e.g., Stemple states on page 980 that "the precise identity of the neurons that develop in this system is unclear." Applicants have carefully reviewed Stemple '92 and have not found a single reference to the ret protein.

Martucciello refers to immunohistochemical study of expression and localization of the RET protein in the intestinal plexuses of patients with Hirschspring's disease. Immunohistochemistry was performed with monoclonal and polyclonal antibodies against the RET protein. Martucciello does not teach or suggest enrichment of individual cell lineages from neural crest stem cells that are RET^+. Furthermore, Martucciello only investigated tissues, not individual cells for the expression of RET protein. A person of skill in the art would not be able to conclude, with any degree of certainty, that RET^+ cells are useful for the purification of neural stem cells from reading Martucciello.

In summary, the combination of Lo, Stemple '92, Stemple '93 and Martucciello do not teach the usefulness of using the RET antigen as a selection marker for neural progenitor cells. The references do not teach this individually, and further, they do not teach this in a combination.

Furthermore, the cited references actually teaches away from the claimed invention. Lo teaches away from the claimed invention because it is, in fact, wrong. In Lo, the authors proposed a scheme for neural cell development in Figure 6 which is "purely speculative" (Lo, legend to Figure 6). In fact, this "purely speculative" proposed neural crest development schematic is wrong. For example, the lineage diagram of Figure 6 shows that the progenitor to glial cells is c-ret-. In fact, Applicants teaches in the instant application that glial progenitors are $c\text{-}ret^+$. See, e.g., instant application, page 32, lines. 17-19. Thus, Lo actually teaches away from the claimed invention. Lo teaches that a c-ret precursor cell is not useful for the production of glial progenitor cells while the claimed invention is directed to a method of selecting a $c\text{-}ret^+$ cell for glial precursors.

For this reason, an artisan reviewing Lo, or Lo in combination with Stemple '92, Stemple '93 and Martucciello would actually be discouraged from using RET as a marker for neural stem cells.

For these reasons, the rejection of claims 4-8 and 12-16 under 35 U.S.C. § 103 as allegedly obvious in view of Lo et al., Stemple '93, Stemple '92 and Martucciello et al. is in error and should be withdrawn.

§ 102

Claims 8, 13, 14 and 16 stand rejected under 35 U.S.C. § 102 as allegedly anticipated by Stemple '92 (Stemple et al Cell 71:973-985, 1992). Applicants traverse.

To be anticipated, each and every claimed element must be disclosed in a single prior reference. Each of the rejected claims (8, 13, 14, and 16) has, as a claim element, the RET protein. Specifically, claim 16 is directed to "A substantially pure population of neural crest derived neural progenitor cells comprising RET protein . . ." Claims 8, 13 and 14, being dependent of claim 16, are also directed to a cell population comprising RET protein. As discussed above for § 103, Applicants have carefully reviewed Stemple '92 and have not found a single reference to the RET protein. Since each and every limitation of the claimed invention is not disclosed in Stemple '92, it is not a proper reference for a § 102 rejection of claims 8, 13, 14 and 16.

Further, Applicant's claimed cells are in fact different from the cells of Stemple '92. Applicants have analyzed cells using the antibody of Stemple '92 and concluded "anti-RET and anti-p75LNGFR antibodies enrich for distinct populations of neural crest-derived cells in the gut." (Specification, page 26, lines 24-26.). For example, 14% of the anti-p75LNGFR antibody isolated cells were MASH+ while 82% of anti-RET antibody isolated cells were MASH+.

Thus, the rejection of claims 8, 13, 14 and 16 under 35 U.S.C. § 102 as allegedly anticipated by Stemple '92 is in error and should be withdrawn.

CONCLUSION

Applicants believe that the claims are in condition for allowance and such disposition is earnestly requested.

Applicants believe no further fee is due at this time; however, the Commissioner is authorized to charge any additional fees that may be due, or to credit any overpayment, to the undersigned's account, Deposit Account No. 50-0311, Reference Number: 18444-502 (Customer Number: 35437).

If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Date: February 18, 2004

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